Amendments to the Specification

Please amend the paragraph at page 7, line 12 as follows:

BRIEF DESCRIPTION OF THE ~~FIGURES~~ DRAWINGS

Please amend the paragraph at page 8, line 14 as follows:

Figures 21 A-D provides SEQ ID NO:37, the coding sequence for a vector encoding an IgM.

Please amend the paragraph at page 8, line 15 as follows:

Figures 22 A-D provides SEQ ID NO:38, the coding sequence for one vector of a two vector system for producing an IgM.

Please amend the paragraph at page 8, line 17 as follows:

Figures 23 A-C provides SEQ ID NO:39, the coding sequence for one vector of a two vector system for producing an IgM.

Please amend the paragraph at page 8, line 19 as follows:

Figures 24 A-C provides SEQ ID NO:40, the coding sequence for a retroviral vector comprising an amplifiable marker (dhfr).

Please amend the paragraph at page 8, line 21 as follows:

Figures 25 A-C provides SEQ ID NO:41, the coding sequence for a retroviral vector comprising an amplifiable marker (gs).

Please amend the paragraph at page 50, line 28 as follows:

In some preferred embodiments, the vectors are engineered to express an immunoglobulin (e.g., IgG, IgA, IgM, IgD, IbgE and sIg). Examples of such vectors are provided in Figures 7-16 (SEQ IN NOs: 4-13). When expression of immunoglobulins with a J chain (e.g., IgM) is desired, different approaches may be utilized. In some embodiments, a single retroviral vector is used. In some embodiments, the J chain is placed under the control of the LTR promoter. In some embodiments, the resulting vector (see Figure 21, SEQ ID NO:37) comprises the following elements in operable association: 5'LTR, MoMLV extended packaging region J chain gene, internal promoter, signal peptide, heavy chain gene, IRES, light chain gene, RNA export element, MoMLV 3' LTR. In other embodiments, two separate retrovectors are used, one for expressing a J chain chain and the other for expressing the heavy and light chains. Representative vectors are provided in Figures 22 (SEQ ID NO:38) and 23 (SEQ ID NO:39). In some embodiments, the heavy/light chain vector is used to make a cell line comprising multiple copies of the vector (e.g., through high multiplicity of infection transduction or serial transduction or a combination of the two). A clonal cell line is then selected and tranduced with the J chain chain vector. In some embodiments, the vector encoding the J chain contains a selectable marker (e.g., blast) that is different from the selectable marker in the heavy/light chain vector (e.g., neo). Individual clonal lines expressing functional IgM are then selected. It will be recognized that the order of transduction can be altered (i.e., the cells can be transduced with J chain vector first, and heavy/light chain vector second).

Please amend the paragraph at page 58, line 12 as follows:

In the experimental disclosure which follows, the following abbreviations apply: M (molar); mM (millimolar); μM (micromolar); nM (nanomolar); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); gm (grams); mg (milligrams); μg (micrograms);pg (picograms); L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); AMP (adenosine 5'-monophosphate); BSA (bovine serum albumin); cDNA (copy or complimentary DNA); CS (calf serum); DNA

(deoxyribonucleic acid); ssDNA (single stranded DNA); dsDNA (double stranded DNA); dNTP (deoxyribonucleotide triphosphate); LH (luteinizing hormone); NIH (National Institues of Health, Besthesda, MD); RNA (ribonucleic acid); PBS (phosphate buffered saline); g (gravity); OD (optical density); HEPES (N-[2-Hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]); HBS (HEPES buffered saline); PBS (phosphate buffered saline); SDS (sodium dodecylsulfate); Tris-HCl (tris[Hydroxymethyl]aminomethane-hydrochloride); Klenow (DNA polymerase I large (Klenow) fragment); rpm (revolutions per minute); EGTA (ethylene glycol-bis(ß-aminoethyl ether) N, N, N', N'-tetraacetic acid); EDTA (ethylenediaminetetracetic acid); bla (ß-lactamase or ampicillin-resistance gene); ORI (plasmid origin of replication); lacI (lac repressor); X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside); ATCC (American Type Culture Collection, ~~Rockville~~ Manassas, VA ~~MD~~); GIBCO/BRL (GIBCO/BRL, Grand Island, NY); Perkin-Elmer (Perkin-Elmer, Norwalk, CT); and Sigma (Sigma Chemical Company, St. Louis, MO).

Please amend the paragraph at page 77, line 25 as follows:

The VSV G-pseudotyped LNBOTDC viruses were concentrated to a high titer by one cycle of ultracentrifugation. However, two cycles can be performed for further concentration. The frozen culture medium collected as described in Example 2 which contained pseudotyped LNBOTDC virus was thawed in a 37°C water bath and was then transferred to Oakridge centrifuge tubes (50 ml ~~Oakridge tubes~~ OAKRIDGE TUBES™ with sealing caps, Nalge Nunc International) previously sterilized by autoclaving. The virus was sedimented in a JA20 rotor (Beckman) at 48,000 x g (20,000 rpm) at 4°C for 120 min. The culture medium was then removed from the tubes in a biosafety hood and the media remaining in the tubes was aspirated to remove the supernatent. The virus pellet was resuspended to 0.5 to 1% of the original volume of culture medium DMEM. The resuspended virus pellet was incubated overnight at 4°C without swirling. The virus pellet could be dispersed with gentle pipetting after the overnight incubation without significant loss of infectious virus. The titer of the virus stock was routinely increased 100- to 300-fold after one round of ultracentrifugation. The efficiency of recovery of infectious virus varied between 30 and 100%.

Please amend the paragraph at page 104, line 12 as follows:

The VSV G-pseudotyped viruses were concentrated to a high titer by one cycle of ultracentrifugation. However, in certain embodiments, two cycles are performed for further concentration. The culture medium collected and filtered as described in Example 26 which contained pseudotyped virus was transferred to Oakridge centrifuge tubes (50 ml ~~Oakridge tubes~~ OAKRIDGE TUBES™ with sealing caps, Nalge Nunc International) previously sterilized by autoclaving. The virus was sedimented in a JA20 rotor (Beckman) at 48,000 x g (20,000 rpm) at 4°C for 120 min. The culture medium was then removed from the tubes in a biosafety hood and the media remaining in the tubes was aspirated to remove the supernatant. The virus pellet was resuspended to 0.5 to 1% of the original volume in 0.1X HBSS. The resuspended virus pellet was incubated overnight at 4°C without swirling. The virus pellet could be dispersed with gentle pipetting after the overnight incubation without significant loss of infectious virus. The titer of the virus stock was routinely increased 100- to 300-fold after one round of ultracentrifugation. The efficiency of recovery of infectious virus varied between 30 and 100%.

Please amend the paragraph at page 95, line 29 as follows:

This example describes the expression of a G-Protein Coupled Receptor protein (GPCR) from a retroviral vector. This example also describes the expression of a signal protein from an IRES as a marker for expression of a difficult to assay protein or a protein that has no assay such as a GPCR. The gene construct (SEQ ID NO: 34; Figure 19) comprises a G-protein-coupled receptor followed by the IRES-signal peptide-antibody light chain cloned into the MCS of pLBCX retroviral backbone. Briefly, a PvuII/PvuII fragment (3057 bp) containing the GPCR-IRES-antibody light chain was cloned into the StuI site of pLBCX. pLBCX contains the EM7 (T7) promoter, ~~Blasticidin~~ BLASTICIDIN™ gene and SV40 polyA in place of the Neomycin resistance gene from pLNCX.

Please amend the paragraph at page 96, line 7 as follows:

The gene construct was used to produce a replication defective retroviral packaging cell line and this cell line was used to produce replication defective retroviral vector. The vector produced from this cell line was then used to infect 293GP cells (human embryonic kidney cells). After infection, the cells were placed under ~~Blasticidin~~ BLASTICIDIN™ selection and single cell ~~Blasticidin~~ BLASTICIDIN™ resistant clones were isolated. The clones were screened for expression of antibody light chain. The top 12 light chain expressing clones were selected. These 12 light chain expressing clones were then screened for expression of the GPCR using a ligand binding assay. All twelve of the samples also expressed the receptor protein. The clonal cell lines and there expression are shown in Table 9.

Please amend the paragraph at page 97, line 10 as follows:

Blast =~~Blasticidin~~ BLASTICIDIN™ resistance gene.

Please amend the paragraph at page 8, line 6 as follows:

Figure 17 provides a graph depicting the INVADER™ Assay gene ratio in CMV promoter cell lines.

Please amend the paragraph at page 8, line 8 as follows:

Figure 18 provides a graph depicting the INVADER™ Assay gene ratio in α-lactalbumin promotor cell lines.

Please amend the paragraph at page 46, line 10 as follows:

In some embodiments, after transfection or transduction, the cells are allowed to multiply, and are then trypsinized and replated. Individual colonies are then selected to provide clonally selected cell lines. In still further embodiments, the clonally selected cell lines are screened by Southern blotting or INVADER™ assay to verify that the desired number of integration events

has occurred. It is also contemplated that clonal selection allows the identification of superior protein producing cell lines. In other embodiments, the cells are not clonally selected following transfection.

Please amend the paragraph at page 48, line 30 as follows:

In other embodiments, nucleic acid encoding the protein of interest is detected. For example, in some embodiments, a PCR assay is performed using primers specific for the protein of interest. In other embodiments, nucleic acid is detected via a hybridization assay (*e.g.*, including, but not limited to, Southern Blot, Northern Blot, INVADER™ Assay (Third Wave Technologies, Madison, WI), TaqMan assay (Applied Biosystems, Foster City, CA), and SNP-IT primer extension assay (Orchid Biosciences, Princeton, NJ).

Please amend the paragraph at page 89, line 5 as follows:

This example describes the relationship of multiplicity of infection, gene copy number, and protein expression. Three DNA assays were developed using the INVADER™ Assay system (Third Wave Technologies, Madison, WI). One of the assays detects a portion of the bovine α-lactalbumin 5' flanking region. This assay is specific for bovine and does not detect the porcine or human α-lactalbumin gene. This assay will detect two copies of the α-lactalbumin gene in all control bovine DNA samples and also in bovine mammary epithelial cells. The second assay detects a portion of the extended packaging region from the MLV virus. This assay is specific for this region and does not detect a signal in the 293 human cell line, bovine mammary epithelial cell line or bovine DNA samples. Theoretically, all cell lines or other samples not infected with MLV should not produce a signal. However, since the 293GP cell line was produced with the extended packaging region of DNA, this cell line gives a signal when the assay is run. From the initial analysis, it appears that the 293GP cell line contains two copies of the extended packing region sequence that are detected by the assay. The final assay is the control assay. This assay detects a portion of the insulin-like growth factor I gene that is identical in bovine, porcine, humans and a number of other species. It is used as a control on

every sample that is run in order to determine the amount of signal that is generated from this sample for a two copy gene. All samples that are tested should contain two copies of the control gene.

Please amend the paragraph at page 91, line 7 as follows:

~~Invader~~ INVADER™ Assay Gene Ratio and Cell Line Protein Production

Please amend the paragraph at page 91, line 20 as follows:

For further analysis of these clonal lines, fifteen CMV clones and fifteen α-LA clones were selected. Five highest expressing, five low expressing and five mid-level expressing lines were chosen. These thirty cell lines were expanded and banked. DNA was isolated from most all of the thirty cell lines. The cell lines were passed into 6 well plates and grown to confluency. Once at confluency, the media was changed every 24 hours and two separate collections from each cell line were assayed for MN14 production. The results of these two assays were averaged and these numbers were used to create Tables 6 and 7 below. DNA from the cell lines was run using the ~~Invader~~ INVADER™ extended packaging region assay and the results are shown below. The Tables show the cell line number, corresponding gene ratio and antibody production.

Please amend the paragraph at page 93, line 12 as follows:

The graphs (Figs. 17 and 18) show the comparison between protein expression and ~~Invader~~ INVADER™ assay gene ratio. The results indicate that there is a direct correlation between invader assay gene ratio and protein production. It also appears that the protein production has not reached a maximum and if cells containing a higher ~~Invader~~ INVADER™ assay gene ratio were produced, higher protein production would occur.

Please amend the paragraph at page 93, line 10 as follows:

~~Invader~~ INVADER™ Assay Gene Ratio and Multiple Cell Line Infections

Please amend the paragraph at page 94, line 10 as follows:

Each of the parent cell lines and the daughter production cell lines were examined for ~~Invader~~ INVADER™ gene ratio using the extended packaging region assay and for protein production. The Bot production cell line, which was generated using the highest titer vector had the highest gene ratio. It also had the highest protein production, again suggesting that gene copy number is proportional to protein production. The YP production cell line also had a higher gene ratio and produced more protein than its parent cell line, also suggesting that increasing gene copy is directly related to increases in protein production. The data is presented in Table 8.

Please amend the paragraph at page 97, line 16 as follows:

This packaging cell line was then used to produce a replication defective retroviral vector arranged as follows. The vector was produced from cells grown in T150 flasks and frozen. The frozen vector was thawed at each infection. For infection # 3 a concentrated solution of vector was used to perform the infection. All other infections were performed using non-concentrated vector. The infections were performed over a period of approximately five months by placing 5 ml of vector/media solution on a T25 flask containing 30% confluent 293 cells. Eight mg/ml of polybrene was also placed in the vector solution during infection. The vector solution was left on the cells for 24 hours and then removed. Media (DMEM with 10% fetal calf serum) was then added to the cells. Cells were grown to full confluency and passaged into a new T25 flask. The cells were then grown to 30% confluency and the infection procedure was repeated. This process was repeated 12 times and is outlined Table 10 below. After infections 1, 3, 6, 9 and 12, cells left over after passaging were used to obtain a DNA sample. The DNA was analyzed using the INVADER™ assay to determine an estimate of the number of vector inserts in the cells after various times in the infection procedure. The results indicate that the number of vector insertions goes up over time with the highest level being after the 12^{th} infection. Since a value of 0.5 is approximately an average of one vector insert copy per cell, after twelve infections the average

vector insert copy has yet to reach two. These data indicates that the average vector copy per cell is a little less that 1.5 copies per cell. Also, there was no real change in gene copy number from infection #6 to infection #9. Furthermore, these data indicate that transfection conducted at a standard low multiplicity of infection fail to introduce more than one copy of the retroviral vector into the cells.

Please amend the paragraph at page 100, line 16 as follows:

Two cell lines that contain gene inserts of the LN-CMV-Bot vector were analyzed for their ability to maintain the vector inserts over a number of passages with and without neomycin selection. The first cell line is a bovine mammary epithelial cell line that contains a low number of insert copies. The second cell line is a 293GP line that contains multiple copies of the vector insert. At the start of the experiment, cell cultures were split. This was at passage 10 for the bovine mammary epithelial cells and passage 8 for the 293GP cells. One sample was continually passaged in media containing the neomycin analog G418, the other culture was continually passaged in media without any antibiotic. Every 3-6 passages, cells were collected and DNA was isolated for determination of gene ratio using the INVADER™ assay. Cell were continually grown and passaged in T25 flasks. The results of the assays are shown below: